PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference MRM/24090WO	FOR FURTHER ACTION See Form PCT/IPEA/416	
International application No. PCT/GB2004/000073	International filing date *(day/month/year)* 12.01.2004	Priority date *(day/month/year)* 10.01.2003
International Patent Classification (IPC) or national classification and IPC A61K35/76, A61P31/04		
Applicant HEALTH PROTECTION AGENCY		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 8 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 6 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☒	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
25.06.2004	03.12.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Pilling, S Telephone No. +49 89 2399-8461

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/000073

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   Description, Pages

   1-37 as originally filed

   Claims, Numbers

   1-40 received on 12.11.2004 with letter of 10.11.2004

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
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Box No. II Priority

1. ☒ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
   - ☒ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
   - ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).
2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.
3. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 32 to 40 with regard to industrial applicability

   because:

   ☒ the said international application, or the said claims Nos. 32-40 relate to the following subject matter which does not require an international preliminary examination (specify):

   see separate sheet

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos.

   ☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

   ☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

   ☐ See separate sheet for further details

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/000073

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-30,32-40
	No:	Claims	31
Inventive step (IS)	Yes:	Claims	1-21,23,24,32-40
	No:	Claims	22,25-31
Industrial applicability (IA)	Yes:	Claims	1-31 (for Claims 32 to 40 see comments under Item III)
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Claims 32 to 40 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no international preliminary examination will be made in respect of these claims in respect of industrial applicability (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

2. The documents cited in the International Search Report (ISR) are consecutively numbered D1 to D7 in the order of their listing. If not indicated otherwise, reference is made to the passages cited in said ISR.

3. The relevant disclosures of each of these documents are summarised as follows;

D1: discloses that bacteriophages have enzymes that degrade exopolysaccharide (EPS) in bacterial biofilms.

D2: discloses that most bacteriophages have polysaccharide degrading enzymes including polysaccharide lyases.

D3: discloses that *Staphylococcus epidermidis* biofilm infections in CSF shunts can be treated with bacteriophage.

D4: discloses that *Escherichia coli* biofilm infections in Robbins devices can be treated with bacteriophage.

D5: discloses that *Pseudomonas aeruginosa* infections of skin grafts can be treated with bacteriophage.

D6: discloses that bacteriophage migration through *Pseudomonas aeruginosa* biofilms may be dependent on enzymatic degradation of alginate.

D7: discloses treatment of *Pseudomonas aeruginosa* infections associated with cystic fibrosis (CF) using alginate lyase. The concomitant use of an antibiotic is also disclosed (see column 5 lines 8 to 12)

Claims 1 to 11, 23 and 24: compositions for treating a bacterial biofilm

4. None of the presently available prior art documents disclose the compositions defined in present Claim 1. Thus, the subject matter of Claims 1 to 11, 23 and 24 appears to be new (Article 33(2) PCT).

5. The closest prior art in respect of Claim 1 appears to be document D7 (see the summary of this document above). The difference between the disclosure of document D7 and the subject matter of the present invention is the present incorporation of a bacteriophage encoding a PL enzyme rather than the use of a PL enzyme *per se* as disclosed in D7. Although there are no direct comparative studies, it appears that the present use of a bacteriophage would enhance the therapeutic effect of the composition by causing lysis of the bacteria in the CF biofilm (see the results set out in present Figures 2 to 4), *i.e.* there is an additional antibacterial effect. There is no suggestion in document D7 that the alginate lyase disclosed therein could be replaced by bacteriophage therapy.

6. Document D6 discloses that alginate lyase aids mobility of bacteriophage through CF biofilms but there are no clear conclusions drawn regarding the application of this finding to therapy of CF patients.

7. None of the remaining prior art documents deal with therapy of CF.

8. Thus, it appears that the subject matter of present Claim 1 cannot be obviously derived from any of the presently available prior art documents when considered either alone or in combination. Thus, the subject matter Claims 1 to 11, 23 and 24 appears to be inventive (Article 33(3) PCT).

Claims 12 to 21 and 32 to 40: uses/methods involving treatment of CF biofilms

9. For the reasoning set out in respect of the previously claimed compositions (see above), it follows that the use of such compositions and methods of using such compositions as defined in Claims 12 to 21 and 32 to 40 must also be considered

novel and inventive

Claims 22 and 25 to 30; bacteriophages comprising a heterologous gene, uses thereof and methods for making them

10. None of the presently available prior art documents disclose a bacteriophage comprising a heterologous gene encoding a PL enzyme. Thus, the subject matter of Claims 22 and 25 to 30 is new (Article 33(2) PCT).

11. It is clearly known that bacteriophages may produce PL (see, for example present page 13 line 30 to page 14 line 2, page 15 lines 21 to 28 , D1 and D2). Document D1 in particular discloses that bacteriophages have enzymes that degrade exopolysaccharide (EPS) in bacterial biofilms. The difference between these known bacteriophages and those of the presently claimed invention, is the introduction of a heterologous gene encoding a first polysaccharide lyase enzyme. On the basis of the present description it appears that the introduction of a heterologous gene encoding a PL enables the bacteriophage to degrade bacterial EPS present in biofilms (see page 14 lines 15 to 18) such as those resulting from opportunistic bacterial infections (see page 14 lines 28 to 31).

12. Since, it is known that the susceptibility of bacterial biofilms to attack is at least partially dependent on polysaccharide degrading enzymes encoded by the bacteriophage (see for example D1), it does not appear inventive to modify a bacteriophage by introducing a heterologous gene encoding a particular polysaccharide degrading enzyme, *i.e.* a PL. The technical effects of this modification, *i.e.* enabling degradation of EPS present in bacterial biofilms such as CF biofilms, would have been wholly predictable on the basis of the teaching of the prior art.

13. Thus, the subject matter of Claim 22 and 25 to 30 is not inventive (Article 33(3) PCT).

Claim 31; methods of identifying a bacteriophage

14. Claim 31 is directed towards a method of identifying a bacteriophage comprising two steps "*a) identifying a bacteriophage that is capable of infecting a bacterial species or strain*" and "*b) confirming that said bacteriophage encodes a*

polysaccharide lyase..Etc". In this regard, the present definition of the intended use of the bacteriophage (treatment of a CF biofilm) cannot be relied upon to clearly characterise a claim directed towards methods of identification of a bacteriophage. Thus, Claim 31 appears to merely amount to a method of determining whether a bacteriophage encodes a PL. It is clearly known that bacteriophages may produce PL (see, for example present page 13 line 30 to page 14 line 2, page 15 lines 21 to 28 , D1 and D2). Thus, the method of Claim 31 cannot be new (Article 33(2) PCT).

Claims

1. A composition for treating a bacterial biofilm, wherein the biofilm is a lung biofilm of a cystic fibrosis patient, comprising a first bacteriophage that is capable of infecting a bacterium within said biofilm, a first polysaccharide lyase enzyme that is capable of degrading a polysaccharide within said biofilm, and a pharmaceutically-acceptable antimicrobial agent.

2. A composition according to Claim 1, wherein the pharmaceutically-acceptable antimicrobial agent is an antibiotic or a defensin.

3. A composition according to Claim 1 or Claim 2, further comprising a DNase.

4. A composition according to any preceding claim, further comprising a second polysaccharide lyase, wherein the first and second polysaccharide lyase are different.

5. A composition according to any preceding claim, wherein the first polysaccharide lyase is encoded by the bacteriophage.

6. A composition according to any previous claim, wherein the bacteriophage encodes one or more of a pharmaceutically-acceptable antimicrobial agent, a DNase, or a second polysaccharide lyase that is different from the first polysaccharide lyase.

7. A composition according to any previous claim, comprising a second bacteriophage, which is different from the first bacteriophage, and wherein the second bacteriophage optionally encodes a second polysaccharide lyase.

8. A composition according to any previous claim, comprising a second pharmaceutically-acceptable antimicrobial agent.

9. A composition according to any preceding claim, wherein the biofilm comprises an opportunistic bacterium, preferably *Pseudomonas aeruginosa* and/or *Burkholderia cepacia*.

10. A composition according to any preceding claim, wherein the phage is a GH phage, preferably GH4 (ECACC Accession No. 02121203), GH6 (ECACC Accession No. 02121202), GH13 (ECACC Accession No. 02121201), or GH14 (ECACC Accession No. 02121204).

11. A composition according to any preceding claim, wherein the first and/or second polysaccharide lyase is an alginate lyase.

12. Use of a first bacteriophage, a first polysaccharide lyase enzyme and a pharmaceutically-acceptable antimicrobial agent, for the manufacture of a medicament for treatment of a biofilm, wherein the biofilm is a lung biofilm in a cystic fibrosis patient, wherein the first bacteriophage is capable of infecting a bacterium within said biofilm, and wherein the first polysaccharide lyase enzyme is capable of degrading a polysaccharide within said biofilm.

13. Use according to Claim 12, wherein the medicament is to be administered in more than one separate dose.

14. Use according to Claim 13, wherein the medicament is to be administered in at least three separate doses.

15. Use according to any of Claims 12-14, wherein following administration the bacterial cell count of the biofilm is reduced by at least one log, preferably by at least three logs.

16. Use according to any of Claims 12-15, wherein the first bacteriophage is to be administered prior to, simultaneously with, or subsequent to the first polysaccharide lyase.

17. Use according to any of Claims 12-16, wherein the first bacteriophage is to be administered prior to, simultaneously with, or subsequent to said pharmaceutically-acceptable antimicrobial agent.

18. Use according to any of Claims 12-17, wherein the first bacteriophage is to be administered prior to, simultaneously with, or subsequent to a second polysaccharide lyase that is different from the first polysaccharide lyase.

19. Use according to any of Claims 12-18, wherein the first bacteriophage is to be administered prior to, simultaneously with, or subsequent to a second bacteriophage that is capable of infecting a bacterium within the biofilm, wherein said second bacteriophage is different from the first bacteriophage.

20. Use according to any of Claims 12-19, wherein the first bacteriophage is a GH bacteriophage encoding a first polysaccharide lyase.

21. Use according to any of Claims 12-20, wherein the bacteriophage comprises a heterologous gene encoding a first polysaccharide lyase enzyme.

22. A GH bacteriophage selected from the group consisting of GH4 (ECACC Accession No. 02121203), GH6 (ECACC Accession No. 02121202), GH13 (ECACC Accession No. 02121201), and GH14 (ECACC Accession No. 02121204).

23. A composition according to any of Claims 1-11, further comprising a second bacteriophage according to Claim 22, wherein the first bacteriophage and second bacteriophage are different.

24. A composition according to any of Claims 1-11 or 23 in the form of an aerosol formulation, comprising one or more of an excipient, surfactant, and/or propellant.

25. Use of a bacteriophage according to Claim 22 or a composition according to Claim 23 or 24, for the manufacture of a medicament for treating a biofilm, wherein the biofilm is a lung biofilm of a cystic fibrosis patient.

26. A method of making a modified bacteriophage capable of degrading a biofilm, wherein the biofilm is a lung biofilm of a cystic fibrosis patient, comprising:-

a) selecting at least one gene encoding a polysaccharide lyase enzyme that degrades a polysaccharide within said biofilm;

b) selecting a bacteriophage that is capable of infecting a bacterial species or strain residing within the biofilm; and

c) introducing at least one of the genes selected in step a) into the bacteriophage nucleic acid.

27. A method according to Claim 26, wherein the bacteriophage is selected from the group consisting of GH4 (ECACC Accession No. 02121203), GH6 (ECACC Accession No. 02121202), GH13 (ECACC Accession No. 02121201), or GH14 (ECACC Accession No. 02121204); or a bacteriophage having accession No. ATCC 12055-B1, ATCC 12055-B2, ATCC 12055-B3, ATCC 14205-B1, ATCC 14206-B1, ATCC 14207-B1, ATCC 14209-B1, ATCC 14210-B1, ATCC 14211-B1, ATCC 14212-B1, ATCC 14213-B1, ATCC 14214-B1, ATCC 15692-B2, ATCC 15692-B3, ATCC 25102-B1, ATCC BAA-26-B1, ATCC BAA-27-B1, ATCC BAA-28-B1, ATCC BAA-28-B2, ATCC BAA-29-B1, ATCC BAA-30-B1, ATCC BAA-31-B1, ATCC BAA-47-B1, ATCC BAA-79-B1, ATCC BAA-81-B1, and ATCC BAA-81-B2.

28. A method according to Claim 26-27, wherein the method further comprises the step of testing the efficacy of the modified bacteriophage against the biofilm *in vitro*.

29. A method according to any of Claims 26-28, wherein the bacteriophage

specifically infects an opportunistic bacterium, preferably *Pseudomonas aeruginosa* and/or *Burkholderia cepacia*.

30. A method according to any of Claims 26-29, wherein said at least one gene encodes an alginate lyase.

31. A method of identifying a bacteriophage for use in treatment of a biofilm, wherein the biofilm is a lung biofilm of a cystic fibrosis patient, comprising:-

a) identifying a bacteriophage that is capable of infecting a bacterial species or strain with said biofilm; and

b) confirming that said bacteriophage encodes a polysaccharide lyase that degrades a polysaccharide within the biofilm.

32. A method of treating a biofilm infection, wherein the biofilm is a lung biofilm in a cystic fibrosis patient, comprising administering to the patient:-
a first bacteriophage capable of infecting a bacterium within said biofilm;
a first polysaccharide lyase enzyme capable of degrading a polysaccharide within said biofilm; and a pharmaceutically-acceptable antimicrobial agent.

33. A method according to Claim 32, comprising:
administering to a patient a composition according to any of Claims 1-11 or 23-24, or a bacteriophage according to Claim 22.

34. A method according to Claim 32 or 33, wherein the composition or bacteriophage is administered in more than one separate dose.

35. A method according to any of Claims 32-34, wherein the composition or bacteriophage is administered in at least three separate doses.

36. A method according to any of Claims 32-35, wherein the first bacteriophage is administered prior to, simultaneously with, or subsequent to the first polysaccharide lyase.

37. A method according to any of Claims 32-36, wherein the first bacteriophage is administered prior to, simultaneously with, or subsequent to said pharmaceutically-acceptable antimicrobial agent.

38. A method according to any of Claims 32-37, wherein the first bacteriophage is administered prior to, simultaneously with, or subsequent to a second polysaccharide lyase that is different from the first polysaccharide lyase.

39. A method according to any of Claims 32-38, wherein the first bacteriophage is administered prior to, simultaneously with, or subsequent to a second bacteriophage that is capable of infecting a bacterium within the biofilm, wherein said second bacteriophage is different from the first bacteriophage.

40. A method according to any of Claims 32-39, wherein administration is to the site of infection.